

WE CLAIM:

sub B' 1. A method of detecting a target gene having restricted expression in a eucaryotic organism, which comprises the steps of:

(i) transforming a eucaryotic cell with a DNA sequence encoding a first indicator component under the control of a promoter having restricted expression;

(ii) transforming the cell of (i) or a descendent of the cell by operably integrating into the cell's genome, DNA lacking a promoter but which comprises a sequence encoding a second indicator component;

(iii) producing tissue or specialized cells from the cell of (ii); and

(iv) monitoring the tissue or specialized cells of (iii) for a detectable indicator resulting from both the first and second indicator components.

2. The method claim 1 wherein the eucaryotic cell is an ES cell.

A<sup>2</sup> 3. The method of claims 1 and 2 wherein the first and second indicator components are inactive fragments or subunits of an enzyme which, when combined, provide an active enzyme detectable by its activity.

A<sup>3</sup> 4. The method of claim 1 or 2 wherein the first and second indicator components are independently detectable or selectable, and the detectable indicator is the presence of both indicator components in a cell.

A<sup>4</sup> 5. The method of claim 1 or 2 wherein the first and second indicator components react in a sequence of reactions which result in a detectable indicator.

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Claim 1

6. The method of ~~any one of claims 1-5~~ which comprises the additional step of isolating DNA endogenous to the eucaryotic cell which flanks integrated DNA comprising the second indicator component.

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7. A method of producing an eucaryotic organism comprising a detectable indicator associated with a target gene having restricted expression, comprising steps (i) and (ii) of claim 1 to produce a transformed cell, followed by the step of growing an eucaryotic organism from said transformed cell.

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8. The method of claim 7 wherein the eucaryotic organism is a non-human mammal and the transformed cell is an ES cell.

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C<sup>1</sup>

9. A DNA construct comprising, in a 5' to 3' direction, a splice acceptor and a sequence encoding an inactive subunit or fragment of an enzyme, wherein said subunit or fragment is active when combined with a further subunit or fragment of an enzyme.

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10. The DNA construct of claim 9 wherein the sequence encodes an alpha or omega fragment of  $\beta$ -galactosidase.

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11. The DNA construct of claim 9 or 10 further comprising an IRES.

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C<sup>2</sup>

12. The combination of:

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(i) a DNA construct for integration into the genome of an eucaryotic cell comprising a sequence encoding a first indicator component under the control of a promoter having restricted expression; and

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(ii) A DNA construct for integration into the genome of a eucaryotic cell, comprising in the 5' to 3' direction, a splice acceptor and a sequence encoding a second

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indicator component and an optional IRES,  
wherein expression of both the first and second indicator  
components in said cell is detectable.

- 5 13. A eucaryotic cells transformed by the combination of  
DNA constructs of claim 12.

14. The cell of claim 13 which is an ES cell.

add B5 >

123456789101112131415161718192021222324252627282930313233343536373839404142434445464748495051525354555657585960616263646566676869707172737475767778798081828384858687888990919293949596979899100